L Number	Hits	Search Text	DB	Time stamp
1	2	6225438.pn.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 11:20
2	103993	yeast	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 11:20
3	450868	polyester	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 11:20
4	62987	"expression vector" or "expression cassette" or "expression plasmid"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 11:20
5	490	yeast and polyester and ("expression vector" or "expression cassette" or "expression plasmid")	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 11:21
6	15254	yeast SAME ("expression vector" or "expression cassette" or "expression plasmid")	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 11:21
7	187	(yeast SAME ("expression vector" or "expression cassette" or "expression plasmid")) and polyester	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 11:32
8	10586	transformation SAME yeast	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 11:24
9	1	(transformation SAME yeast) SAME polyester	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 11:25
10	10935	polyhydroxyalkanoate or hydroxyalkanoic or hydroxybutyric or hydroxyhexanoic	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 11:33
11	84	(polyhydroxyalkanoate or hydroxyalkanoic or hydroxybutyric or hydroxyhexanoic) SAME yeast	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 11:33
12	16	((polyhydroxyalkanoate or hydroxyalkanoic or hydroxybutyric or hydroxyhexanoic) SAME yeast) and ("expression vector" or "expression cassette" or "expression plasmid")	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:24
13	16	3HH WITH 3HB	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:30
14	182478	yokomizo.in. or fukuchi.in. or osakada.in. or matsumoto.in. or takagi.in. or ohta.in. or ota.in.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:31
15	4005	(yokomizo.in. or fukuchi.in. or osakada.in. or matsumoto.in. or takagi.in. or ohta.in. or ota.in.) and polyester	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:31
16	19	((yokomizo.in. or fukuchi.in. or osakada.in. or matsumoto.in. or takagi.in. or ohta.in. or ota.in.) and polyester) and yeast	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:32
17	9420	riken.as.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:32

18	1	riken.as. and polyester and yeast	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:35
19	1	"WO 00/43525"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:36
20	2505	lipolytica or maltosa or caviae	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:37
21	673	ALK1 or ALK3 or XPR2	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:37
22	159	enoyl-CoA WITH hydrata$	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:38
23	726	PHA WITH synth$	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:38
24	97	(lipolytica or maltosa or caviae) and polyester and ("expression vector" or "expression cassette" or "expression plasmid")	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:39
25	17	((lipolytica or maltosa or caviae) and polyester and ("expression vector" or "expression cassette" or "expression plasmid")) and (3HH or 3HB)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:39
26	25	(ALK1 or ALK3 or XPR2) SAME (lipolytica or maltosa or caviae)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:40
27	1	((ALK1 or ALK3 or XPR2) SAME (lipolytica or maltosa or caviae)) and (3hh or 3hb)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:40
28	72	(PHA WITH synth$) and (lipolytica or maltosa or caviae)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:41
29	50	((PHA WITH synth$) and (lipolytica or maltosa or caviae)) and (3HH or 3HB)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:43
30	0	6635782.in.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:43
31	2	6635782.pn.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:44
32	44	(((PHA WITH synth$) and (lipolytica or maltosa or caviae)) and (3HH or 3HB)) and yeast	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:46
33	22	(PHA WITH synth$) SAME caviae	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:47
34	13	((PHA WITH synth$) SAME caviae) and yeast	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:53
35	37268	|435/254.11|435/254.2|536/23.1|536/23.4|435/[illegible]|435/[illegible]|435/471|435/	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:56

36	269	|435/254.11|435/254.2|536/23.1|536/23.4|43[illegible]|435/[illegible]|435/471|435/ and yeast and polyester	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 12:56
37	23	(|435/254.11|435/254.2|536/23.1|536/23.4|4[illegible]1|435/[illegible]2|435/471|435 and yeast and polyester) and (3hh or 3hb)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 13:00
38	1094	"modified codon" or "altered codon" or "mutated codon" or (CTG WITH mutated) or (CTG WITH altered) or (CTG WITH modified)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 13:01
39	5	("modified codon" or "altered codon" or "mutated codon" or (CTG WITH mutated) or (CTG WITH altered) or (CTG WITH modified)) and (PHA WITH synth$)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 13:02
40	16	("modified codon" or "altered codon" or "mutated codon" or (CTG WITH mutated) or (CTG WITH altered) or (CTG WITH modified)) and yeast and polyester and ("expression vector" or "expression cassette" or "expression plasmid")	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 13:03
41	1	("modified codon" or "altered codon" or "mutated codon" or (CTG WITH mutated) or (CTG WITH altered) or (CTG WITH modified)) and caviae and (polyester SAME enzyme)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 13:03
42	1	("modified codon" or "altered codon" or "mutated codon" or (CTG WITH mutated) or (CTG WITH altered) or (CTG WITH modified)) and (3HH or 3HB or 4HH or 4HB)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 13:09
43	10	("modified codon" or "altered codon" or "mutated codon" or (CTG WITH mutated) or (CTG WITH altered) or (CTG WITH modified)) and aeromonas	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 13:09
44	281	("modified codon" or "altered codon" or "mutated codon" or (CTG WITH mutated) or (CTG WITH altered) or (CTG WITH modified)) and biosynthesis	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 13:09
45	216	(("modified codon" or "altered codon" or "mutated codon" or (CTG WITH mutated) or (CTG WITH altered) or (CTG WITH modified)) and biosynthesis) and (polymer or copolymer or co-polymer)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 13:10
46	50	((("modified codon" or "altered codon" or "mutated codon" or (CTG WITH mutated) or (CTG WITH altered) or (CTG WITH modified)) and biosynthesis) and (polymer or copolymer or co-polymer)) and hydroxy	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 13:12
47	0	((("modified codon" or "altered codon" or "mutated codon" or (CTG WITH mutated) or (CTG WITH altered) or (CTG WITH modified)) and biosynthesis) and (polymer or copolymer or co-polymer)) and (aeromonas or caviae)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 13:12
48	10	("modified codon" or "altered codon" or "mutated codon" or (CTG WITH mutated) or (CTG WITH altered) or (CTG WITH modified)) and (aeromonas or caviae)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 13:12
-	0	ykomizo.in	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 11:10
-	0	yokomizo.in	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/07/20 18:11
-	0	"WO" WITH "0188144"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/07/20 18:11

-	0	"WO 0188144"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/07/20 18:19
-	21430	doi.in.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/07/20 18:19
-	164	doi.in. and fukui.in.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/07/20 18:20
-	13	(doi.in. and fukui.in.) and "polyester"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/07/20 18:20

	Document ID	Title
1	US 20040146998 A1	Transformant and process for producing polyester by using the same
2	US 20040106176 A1	Polyhydroxyalkanoate production by coenzyme A-dependent aldehyde dehydrogenase pathways
3	US 20040023347 A1	Polyhydroxyalkanoate production from polyols
4	US 20030233677 A1	Modification of fatty acid metabolism in plants
5	US 20030191146 A1	Novel antimicrobial activity of gemfibrozil and related compounds and derivatives and metabolites thereof
6	US 20030167532 A1	OAR polynucleotides, polypeptides and their use in PHA production in plants
7	US 20030073019 A1	Binder resin containing polyhydroxyalkanoate, toner containing the binder resin, and image-forming method and image-forming apparatus which make use of the toner
8	US 20020142406 A1	Polyhydroxyalkanoate production from polyols
9	US 6586658 B1	Modification of fatty acid metabolism in plants
10	US 6576450 B2	Polyhydroxyalkanoate production from polyols
11	US 6531291 B1	Antimicrobial activity of gemfibrozil and related compounds and derivatives and metabolites thereof

	Document ID	Title
12	US 6475734 B1	Polyhydroxyalkanoate synthase genes
13	US 6329183 B1	Polyhydroxyalkanoate production from polyols

	Document ID	Title
1	US 20040146998 A1	Transformant and process for producing polyester by using the same
2	US 20040106176 A1	Polyhydroxyalkanoate production by coenzyme A-dependent aldehyde dehydrogenase pathways
3	US 20040023347 A1	Polyhydroxyalkanoate production from polyols
4	US 20030233677 A1	Modification of fatty acid metabolism in plants
5	US 20030191146 A1	Novel antimicrobial activity of gemfibrozil and related compounds and derivatives and metabolites thereof
6	US 20030167532 A1	OAR polynucleotides, polypeptides and their use in PHA production in plants
7	US 20030073019 A1	Binder resin containing polyhydroxyalkanoate, toner containing the binder resin, and image-forming method and image-forming apparatus which make use of the toner
8	US 20020142406 A1	Polyhydroxyalkanoate production from polyols
9	US 6586658 B1	Modification of fatty acid metabolism in plants
10	US 6576450 B2	Polyhydroxyalkanoate production from polyols
11	US 6531291 B1	Antimicrobial activity of gemfibrozil and related compounds and derivatives and metabolites thereof

	Document ID	Title
12	US 6475734 B1	Polyhydroxyalkanoate synthase genes
13	US 6329183 B1	Polyhydroxyalkanoate production from polyols

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  FILE 'MEDLINE, EMBASE, BIOSIS' ENTERED AT 19:21:59 ON 03 AUG 2004
L1          9305 S POLYESTER
L2        269203 S YEAST
L3         46122 S "EXPRESSION CASSETTE" OR "EXPRESSION VECTOR" OR "EXPRESSION P
L4         31657 S POLYHYDROXYALKANOATE OR HYDROXYALKANOIC OR HYDROXYBUT? OR HYD
L5             0 S LIPOLYTICA SAME YARROWIA
L6          1822 S YARROWIA
L7           624 S CANDIDA (P) MALTOSA
L8          1771 S L6 (P) LIPOLYTICA
L9           422 S ALK3 OR ALK1 OR XPR2
L10          607 S POLY (S) HYDROXYALKANOAT?
L11         3233 S PHA (S) SYNTHES?
L12         1756 S CAVIAE
L13            0 S L1 AND L2 AND L3
L14            2 S L1 AND L2 AND L4
L15            2 DUP REM L14 (0 DUPLICATES REMOVED)
L16           27 S L2 AND L1
L17           19 DUP REM L16 (8 DUPLICATES REMOVED)
L18           14 S L17 NOT PY>=2002
L19            0 S L8 AND L1
L20            0 S L7 AND L1
L21            0 S L10 AND L6
L22            0 S L9 AND L1
L23           79 S L11 AND L1
L24            1 S L23 AND L2
L25        87616 S CODON
L26      1233259 S ALTERED OR MODIFIED OR SUBSTITUTED OR MUTATED OR REPLACED
L27         5256 S L25 (S) L26
L28            0 S L27 AND L1
L29            5 S L27 AND L4
L30            3 DUP REM L29 (2 DUPLICATES REMOVED)
L31         4641 S "CODON USAGE"
L32           15 S L31 (P) "CTG"
L33            5 DUP REM L32 (10 DUPLICATES REMOVED)
L34          246 S L2 AND L4
L35          209 S L34 NOT PY>=2002
L36          134 DUP REM L35 (75 DUPLICATES REMOVED)
L37            0 S L36 AND (POLYESTER (P) SYNTHESIS)
L38            2 S L11 AND L36
L39           20 S L11 AND L12
L40            7 DUP REM L39 (13 DUPLICATES REMOVED)

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L40 ANSWER 1 OF 7 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2003387009 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12902277
TITLE: Alteration of chain length substrate specificity of Aeromonas **caviae** R-enantiomer-specific enoyl-coenzyme A hydratase through site-directed mutagenesis.
AUTHOR: Tsuge Takeharu; Hisano Tamao; Taguchi Seiichi; Doi Yoshiharu
CORPORATE SOURCE: Department of Innovative and Engineered Materials, Tokyo Institute of Technology, Midori-ku, Yokohama 226-8502, Japan.. ttsuge@iem.titech.ac.jp
SOURCE: Applied and environmental microbiology, (2003 Aug) 69 (8) 4830-6.
Journal code: 7605801. ISSN: 0099-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200309
ENTRY DATE: Entered STN: 20030820
Last Updated on STN: 20030923
Entered Medline: 20030922

AB Aeromonas **caviae** R-specific enoyl-coenzyme A (enoyl-CoA) hydratase (PhaJ(Ac)) is capable of providing (R)-3-hydroxyacyl-CoA with a chain length of four to six carbon atoms from the fatty acid beta-oxidation pathway for polyhydroxyalkanoate (**PHA**) **synthesis**. In this study, amino acid substitutions were introduced into PhaJ(Ac) by site-directed mutagenesis to investigate the feasibility of altering the specificity for the acyl chain length of the substrate. A crystallographic structure analysis of PhaJ(Ac) revealed that Ser-62, Leu-65, and Val-130 define the width and depth of the acyl-chain-binding pocket. Accordingly, we targeted these three residues for amino acid substitution. Nine single-mutation enzymes and two double-mutation enzymes were generated, and their hydratase activities were assayed in vitro by using trans-2-octenoyl-CoA (C(8)) as a substrate. Three of these mutant enzymes, L65A, L65G, and V130G, exhibited significantly high activities toward octenoyl-CoA than the wild-type enzyme exhibited. PHA formation from dodecanoate (C(12)) was examined by using the mutated PhaJ(Ac) as a monomer supplier in recombinant Escherichia coli LS5218 harboring a PHA synthase gene from Pseudomonas sp. strain 61-3 (phaC1(Ps)). When L65A, L65G, or V130G was used individually, increased molar fractions of 3-hydroxyoctanoate (C(8)) and 3-hydroxydecanoate (C(10)) units were incorporated into PHA. These results revealed that Leu-65 and Val-130 affect the acyl chain length substrate specificity. Furthermore, comparative kinetic analyses of the wild-type enzyme and the L65A and V130G mutants were performed, and the mechanisms underlying changes in substrate specificity are discussed.

L40 ANSWER 2 OF 7 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2003176668 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12694916
TITLE: Enhanced production of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) via manipulating the fatty acid beta-oxidation pathway in E. coli.
AUTHOR: Lu Xiaoyun; Zhang Jinyu; Wu Qiong; Chen Guo-Qiang
CORPORATE SOURCE: Department of Biological Science and Biotechnology, Tsinghua University, Beijing 100084, PR China.
SOURCE: FEMS microbiology letters, (2003 Apr 11) 221 (1) 97-101.
Journal code: 7705721. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: (EVALUATION STUDIES)

Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200306
ENTRY DATE: Entered STN: 20030417
Last Updated on STN: 20030617
Entered Medline: 20030616

AB Acyl-CoA dehydrogenase gene (yafH) of Escherichia coli was expressed together with polyhydroxyalkanoate synthase gene (phaC(Ac)) and (R)-enoyl-CoA hydratase gene (phaJ(Ac)) from Aeromonas **caviae**. The expression plasmids were introduced into E. coli JM109, DH5 alpha and XL1-blue, respectively. Compared with the strains harboring only phaC(Ac) and phaJ(Ac), all recombinant E. coli strains harboring yafH, phaC(Ac) and phaJ(Ac) accumulated at least four times more poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx). Cell dry weights produced by all recombinants containing yafH were also considerably higher than that without yafH. The addition of acrylic acid which serves as inhibitor for beta-oxidation and may lead to more precursor supply for **PHA synthesis** did not result in improved PHBHHx production compared with that of the overexpression of yafH. It appeared that the overexpression of acyl-CoA dehydrogenase gene (yafH) enhanced the supply of enoyl-CoA which is the substrate of (R)-enoyl-CoA hydratase. With the enhanced precursor supply, the recombinants accumulated more PHBHHx.

L40 ANSWER 3 OF 7 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2002265536 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11976116
TITLE: Enhanced accumulation and changed monomer composition in polyhydroxyalkanoate (PHA) copolyester by in vitro evolution of Aeromonas **caviae** PHA synthase.
AUTHOR: Kichise Tomoyasu; Taguchi Seiichi; Doi Yoshiharu
CORPORATE SOURCE: Polymer Chemistry Laboratory, RIKEN Institute, 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan.
SOURCE: Applied and environmental microbiology, (2002 May) 68 (5) 2411-9.
Journal code: 7605801. ISSN: 0099-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200207
ENTRY DATE: Entered STN: 20020514
Last Updated on STN: 20020713
Entered Medline: 20020712

AB By in vitro evolution experiment, we have first succeeded in acquiring higher active mutants of a synthase that is a key enzyme essential for bacterial **synthesis** of biodegradable polyester, polyhydroxyalkanoate (**PHA**). Aeromonas **caviae** FA440 synthase, termed PhaC(Ac), was chosen as a good target for evolution, since it can **synthesize** a **PHA** random copolyester of 3-hydroxybutyrate and 3-hydroxyhexanoate [P(3HB-co-3HHx)] that is a tough and flexible material compared to polyhydroxybutyrate (PHB) homopolyester. The in vitro enzyme evolution system consists of PCR-mediated random mutagenesis targeted to a limited region of the phaC(Ac) gene and screening mutant enzymes with higher activities based on two types of polyester accumulation system by using Escherichia coli for the synthesis of PHB (by JM109 strain) (S. Taguchi, A. Maehara, K. Takase, M. Nakahara, H. Nakamura, and Y. Doi, FEMS Microbiol. Lett. 198:65-71, 2001) and of P(3HB-co-3HHx) [by LS5218 [fadR601 atoC(Con)] strain]. The expression vector for the phaC(Ac) gene, together with monomer-supplying enzyme genes, was designed to synthesize PHB homopolyester from glucose and P(3HB-co-3HHx) copolyester from dodecanoate. Two evolved mutant enzymes, termed E2-50 and T3-11, screened through the evolution system

exhibited 56 and 21% increases in activity toward 3HB-coenzyme A, respectively, and consequently led to enhanced accumulation (up to 6.5-fold content) of P(3HB-co-3HHx) in the recombinant LS5218 strains. Two single mutations in the mutants, N149S for E2-50 and D171G for T3-11, occurred at positions that are not highly conserved among the PHA synthase family. It should be noted that increases in the 3HHx fraction (up to 16 to 18 mol%) were observed for both mutants compared to the wild type (10 mol%).

L40 ANSWER 4 OF 7 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2000179659 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10713420
TITLE: Molecular cloning of two (R)-specific enoyl-CoA hydratase genes from Pseudomonas aeruginosa and their use for polyhydroxyalkanoate synthesis.
AUTHOR: Tsuge T; Fukui T; Matsusaki H; Taguchi S; Kobayashi G; Ishizaki A; Doi Y
CORPORATE SOURCE: Division of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Science, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka, Japan.
SOURCE: FEMS microbiology letters, (2000 Mar 15) 184 (2) 193-8. Journal code: 7705721. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 20000512
Last Updated on STN: 20000512
Entered Medline: 20000428

AB Two Pseudomonas aeruginosa genes, termed phaJ1(Pa) and phaJ2(Pa), homologous to the Aeromonas **caviae** (R)-specific enoyl-CoA hydratase gene (phaJ(Ac)) were cloned using a PCR technique to investigate the monomer-supplying ability for polyhydroxyalkanoate (**PHA**) **synthesis** from beta-oxidation cycle. Two expression plasmids for phaJ1(Pa) and phaJ2(Pa) were constructed and introduced into Escherichia coli DH5alpha strain. The recombinants harboring phaJ1(Pa) or phaJ2(Pa) showed high (R)-specific enoyl-CoA hydratase activity with different substrate specificities, that is, specific for short chain-length enoyl-CoA or medium chain-length enoyl-CoA, respectively. In addition, co-expression of these two hydratase genes with PHA synthase gene in E. coli LS5218 resulted in the accumulation of PHA up to 14-29 wt% of cell dry weight from dodecanoate as a sole carbon source. It has been suggested that phaJ1(Pa) and phaJ2(Pa) products have the monomer-supplying ability for **PHA synthesis** from beta-oxidation cycle.

L40 ANSWER 5 OF 7 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 1999346699 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10418145
TITLE: Co-expression of 3-ketoacyl-ACP reductase and polyhydroxyalkanoate synthase genes induces PHA production in Escherichia coli HB101 strain.
AUTHOR: Taguchi K; Aoyagi Y; Matsusaki H; Fukui T; Doi Y
CORPORATE SOURCE: Polymer Chemistry Laboratory, Institute of Physical and Chemical Research (RIKEN), Saitama, Japan.
SOURCE: FEMS microbiology letters, (1999 Jul 1) 176 (1) 183-90. Journal code: 7705721. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199908
ENTRY DATE: Entered STN: 19990827

Last Updated on STN: 19990827
Entered Medline: 19990813

AB The Escherichia coli 3-ketoacyl-ACP reductase gene (fabGEc) was cloned using a PCR technique to investigate the metabolic link between fatty acid metabolism and polyhydroxyalkanoate (PHA) production. Three plasmids respectively harboring fabGEc and the poly-3-hydroxyalkanoate synthesis genes phaCAc and phaC1Ps from Aeromonas **caviae** and Pseudomonas sp. 61-3 respectively were constructed and introduced into E. coli HB101 strain. On a two-stage cultivation using dodecanoate as the sole carbon source, recombinant E. coli HB101 strains harboring fabGEc and phaC genes accumulated PHA copolymers (about 8 wt% of dry cell weight) consisting of several (R)-3-hydroxyalkanoate units of C4, C6, C8, and C10. It has been suggested that overexpression of the fabGEc gene leads to the supply of (R)-3-hydroxyacyl-CoA for **PHA synthesis** via fatty acid degradation.

L40 ANSWER 6 OF 7 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 97386422 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9244271
TITLE: Cloning and analysis of the poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) biosynthesis genes of Aeromonas **caviae**.
AUTHOR: Fukui T; Doi Y
CORPORATE SOURCE: Polymer Chemistry Laboratory, Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama, Japan.
SOURCE: Journal of bacteriology, (1997 Aug) 179 (15) 4821-30. Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-D88825
ENTRY MONTH: 199710
ENTRY DATE: Entered STN: 19971105
Last Updated on STN: 19971105
Entered Medline: 19971023

AB A 5.0-kbp EcoRV-EcoRI restriction fragment was cloned and analyzed from genomic DNA of Aeromonas **caviae**, a bacterium producing a copolyester of (R)-3-hydroxybutyrate (3HB) and (R)-3-hydroxyhexanoate (3HHx) [P(3HB-co-3HHx)] from alkanoic acids or oils. The nucleotide sequence of this region showed a 1,782-bp poly (3-hydroxyalkanoate) (PHA) synthase gene (phaC(Ac) [i.e., the phaC gene from A. **caviae**]) together with four open reading frames (ORF1, -3, -4, and -5) and one putative promoter region. The cloned fragments could not only complement **PHA**-negative mutants of Alcaligenes eutrophus and Pseudomonas putida, but also confer the ability to **synthesize** P(3HB-co-3HHx) from octanoate or hexanoate on the mutants' hosts. Furthermore, coexpression of ORF1 and ORF3 genes with phaC(Ac) in the A. eutrophus mutant resulted in a decrease in the polyester content of the cells. Escherichia coli expressing ORF3 showed (R)-enoyl-coenzyme A (CoA) hydratase activity, suggesting that (R)-3-hydroxyacyl-CoA monomer units are supplied via the (R)-specific hydration of enoyl-CoA in A. **caviae**. The transconjugant of the A. eutrophus mutant expressing only phaC(Ac) effectively accumulated P(3HB-co-3HHx) up to 96 wt% of the cellular dry weight from octanoate in one-step cultivation.

L40 ANSWER 7 OF 7 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 7
ACCESSION NUMBER: 97356372 EMBASE
DOCUMENT NUMBER: 1997356372
TITLE: Biosynthesis of poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxy-heptanoate) terpolymers by recombinant Alcaligenes eutrophus.

AUTHOR: Fukui T.; Kichise T.; Yoshida Y.; Doi Y.
CORPORATE SOURCE: T. Fukui, Polymer Chemistry Laboratory, Institute Physical Chemical Research, Hirosawa 2-1, Wako-shi, Saitama 351-01, Japan. ydoi@postman.riken.go.jp
SOURCE: Biotechnology Letters, (1997) 19/11 (1093-1097).
Refs: 14
ISSN: 0141-5492 CODEN: BILED3
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Recombinant strains of Alcaligenes eutrophus harboring the polyhydroxyalkanoate (**PHA**) synthase gene of Aeromonas **caviae synthesized** poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyheptanoate) terpolymers from alkanoic acids of odd carbon numbers. The results indicated the specificity of **PHA** synthase of A. **caviae** toward 3-hydroxyalkanoate units from C4 to C7. The composition of the polyesters formed varied as the carbon numbers of the alkanoic acids fed increased.

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